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1
Met Ala Leu Pro Phe Ala Leu Leu Met Ala Leu Val Val Leu Ser Cys Lys Ser Ser Cys
ATG GCT TTG CCT TTT GCT TTA CTG ATG GCC CTG GTG GTG CTC AGC TGC AAG TCA AGC TGC

21
Ser Leu Asp Cys Asp Leu Pro Gln Thr His Ser Leu Gly His Arg Arg Thr Met Met Leu
TCT CTG GAC TGT GAT CTG CCT CAG ACC CAC AGC CTG GGT CAC AGG AGG ACC ATG ATG CTC

41
Leu Ala Gln Met Arg Arg Ile Ser Leu Phe Ser Cys Leu Lys Asp Arg His Asp Phe Arg
CTG GCA CAA ATG AGG AGA ATC TCT CTT TTC TCC TGT CTG AAG GAC AGA CAT GAC TTC AGA

61
Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Glu Ala Ile Ser Val Leu
TTT CCC CAG GAG GAG TTT GAT GGC AAC CAG TTC CAG AAG GCT GAA GCC ATC TCT GTC CTC

81
His Glu Val Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Val Ala Trp
CAT GAG GTG ATT CAG CAG ACC TTC AAT CTC TTC AGC ACA AAG GAC TCA TCT GTT GCT TGG

101
Asp Glu Arg Leu Leu Asp Lys Leu Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
GAT GAG AGG CTT CTA GAC AAA CTC TAT ACT GAA CTT TAC CAG CAG CTG AAT GAC CTG GAA

121
Ala Cys Val Met Gln Glu Val Trp Val Gly Gly Thr Pro Leu Met Asn Glu Asp Ser Ile
GCC TGT GTG ATG CAG GAG GTG TGG GTG GGA GGG ACT CCC CTG ATG AAT GAG GAC TCC ATC

141
Leu Ala Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
CTG GCT GTG AGA AAA TAC TTC CAA AGA ATC ACT CTC TAC CTG ACA GAG AAA AAG TAC AGC

161
Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Ser Ser Arg Asn
CCT TGT GCC TGG GAG GTT GTC AGA GCA GAA ATC ATG AGA TCC TTC TCT TCA TCA AGA AAC

181
Leu Gln Glu Arg Leu Arg Arg Lys Glu
TTG CAA GAA AGG TTA AGG AGG AAG GAA
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(57) Abstract

New polypeptide, called IFN-α61, produced by *E. coli* transformed with a newly isolated and characterized human IFN-α gene. The polypeptide exhibits interferon activities such as antiviral activity, cell growth regulation, and regulation of production of cell-produced substances.

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INTERFERON ALPHA 61

Description

Technical Field

The invention is in the field of biotechnology. More particularly it relates to a polypeptide having interferon (IFN) activity, DNA that codes for the polypeptide, a recombinant vector that includes the DNA, a host organism transformed with the recombinant vector that produces the polypeptide, pharmaceutical compositions containing the polypeptide, and therapeutic methods employing the polypeptide.

Background Art

IFNs are proteins with antiviral, immunomodulatory, and antiproliferative activities produced by mammalian cells in response to a variety of inducers (see Stewart, W.E., The Interferon System, Springer-Verlag, New York, 1979). The activity of IFN is largely species specific (Colby, C., and Morgan, M. J., Ann. Rev. Microbiol. 25:333-360 (1971) and thus only human IFN can be used for human clinical studies. Human IFNs are classified into three groups, α, β, and γ, (Nature, 286:110, (1980)). The human IFN-α genes compose a multigene family sharing 85%-95% sequence homology (Goeddel, D. V., et al, Nature 290:20-27 (1981) Nagata, S., et al, J. Interferon Research 1:333-336 (1981)). Several of the IFN-α genes have been cloned and expressed in E.coli (Nagata, S., et

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al, Nature 284:316-320 (1980); Goeddel, D. V., et al, Nature 287:411-415 (1980); Yelverton, E., et al, Nucleic Acids Research, 9:731-741, (1981); Streuli, M., et al, Proc Nat Acad Sci (USA), 78:2848-2852. The resulting polypeptides have been purified and tested for biological activities associated with partially purified native human IFNs and found to possess similar activities. Accordingly such polypeptides are potentially useful as antiviral, immunomodulatory, or antiproliferative agents.

A principal object of the present invention is to provide a polypeptide having interferon activity that is produced by an organism transformed with a newly isolated and newly characterized IFN-α gene. This polypeptide is sometimes referred to herein as IFN-α61. Other objects of the invention are directed to providing the compositions and organisms that are used to produce this polypeptide and to therapeutic compositions and methods that use this polypeptide as an active ingredient.

Disclosure of the Invention

One aspect of the invention is a polypeptide having interferon activity and comprising the amino acid sequence:

CysAspLeuProGln ThrHisSerLeuSer AsnArgArgThrLeu MetIleMetAlaGln
MetGlyArgIleSer ProPheSerCysLeu LysAspArgHisAsp PheGlyPheProGln
GluGluPheAspGly AsnGlnPheGlnLys AlaGlnAlaIleSer ValLeuHisGluMet
IleGlnGlnThrPhe AsnLeuPheSerThr LysAspSerSerAla ThrTrpAspGluThr
LeuLeuAspLysPhe TyrThrGluLeuTyr GlnGlnLeuAsnAsp LeuGluAlaCysMet
MetGlnGluValGly ValGluAspThrPro LeuMetAsnValAsp SerIleLeuThrVal
ArgLysTyrPheGln ArgIleThrLeuTyr LeuThrGluLysLys TyrSerProCysAla
TrpGluValValArg AlaGluIleMetArg SerPheSerLeuSer AlaAsnLeuGlnGlu
ArgLeuArgArgLys Glu

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A second aspect of the invention is a DNA unit or fragment comprising a nucleotide sequence that encodes the above described polypeptide.

A third aspect of the invention is a cloning vehicle or vector that includes the above described DNA.

A fourth aspect of the invention is a host organism that is transformed with the above described cloning vehicle and that produces the above described polypeptide.

A fifth aspect of the invention is a process for producing the above described polypeptide comprising cultivating said transformed host organism and collecting the polypeptide from the resulting culture.

Another aspect of the invention is a pharmaceutical composition having interferon activity comprising an effective amount of the above described polypeptide admixed with a pharmaceutically acceptable carrier.

Still another aspect of the invention is a method of providing interferon therapy to a human comprising administering a therapeutically effective amount of the above described polypeptide to the human.

Brief Description of the Drawings

Figure 1 is a partial restriction map which shows the two XhoII restriction sites that produce a homologous 260 base pair DNA fragment from the IFN-α1 and IFN-α2 structural genes. Data for this map are from Streuli, M., et al Science, 209:1343-1347 (1980).

Figure 2 depicts the sequencing strategy used to obtain the complete DNA sequence of the IFN-α61 gene coding region. Bacteriophage mp7:α61-1

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DNA served as the template for sequences obtained with primers A, H and F and bacteriophage mp7:α61-2 DNA was the template for sequences obtained with primers E and G. The crosshatched area of the gene depicts the region that encodes the 23 amino acid signal polypeptide and the open box depicts the region that encodes the mature polypeptide. The scale, in base pairs, is numbered with 0 representing the ATG start codon of preinterferon. The arrows indicate the direction and extent of sequencing with each primer.

Figure 3 is the nucleotide sequence of the structural gene coding for IFN-α61 including some of the flanking 5'- and 3'- noncoding regions of the gene. The region coding for preinterferon and the mature polypeptide begins with the ATG codon at position 92 and terminates with the TGA codon at position 659.

Figure 4 is a partial restriction map of the coding region of the IFN-α61 gene. The crosshatching represents the region that encodes the 23 amino acid signal peptide and the open box represents the gene coding sequence for the mature polypeptide. The scale, in base pairs, is numbered with 0 representing the ATG start codon of preinterferon.

Figure 5 shows the amino acid sequence of the 23 amino acid signal polypeptide and the 166 amino acid mature IFN-α61 coded for by the gene depicted in Figure 3. The 189 amino acid sequence is displayed above the corresponding nucleotide sequence. Amino acid 24, cysteine, is the first amino acid of the mature IFN-α61 protein.

Figure 6 is the DNA sequence of the E. coli trp promoter and the gene of Figure 3 which was inserted between the EcoRI and HindIII sites of the

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plasmid pBW11. The amino acid sequence of Figure 5 is written above the corresponding DNA sequence and the location of the restriction sites used in the construction of the expression plasmid are indicated.

Figure 7 is a diagram of the expression plasmid, pGW20.

Modes for Carrying Out the Invention

In general terms IFN-α61 was made by identifying and isolating the IFN-α61 gene by screening a library of human genomic DNA with an appropriate IFN-α DNA probe, constructing a vector containing the IFN-α61 gene, transforming microorganisms with the vector, cultivating transformants that express IFN-α61 and collecting IFN-α61 from the culture. A preferred embodiment of this procedure is described below.

DNA Probe Preparation

Total cytoplasmic RNA was extracted from human lymphoblastoid cells, Namalwa, which had been induced for IFN production by pretreatment with 5-bromodeoxyuridine (Tovey, M.G., et al, Nature 267:455-457 (1977)) and Newcastle Disease Virus (NDV). The poly(A) (polyadenylic acid)-containing messenger RNA (mRNA) was isolated from total RNA by chromatography on oligo(dT)-cellulose (type 3 from Collaborative Research; Aviv, H., and Leder, P., Proc Natl Acad Sci (USA), 69:1408-1412, (1972)) and enriched for IFN mRNA by density gradient centrifugation on 5%-20% sucrose gradients. Fractions containing IFN mRNA were identified by translating the mRNA by microinjecting aliquots of each fraction into Xenopus oocytes and determining the IFN activity of the products of the translations according to a method

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described by Colman, A., and Morser, J., Cell, 17:517-526 (1979).

The Namalwa cell human IFN enriched mRNA was used to construct complementary DNA (cDNA) clones in *E. coli* by the G/C tailing method using the PstI site of the cloning vector pBR322 (Bolivar, F., et al, Gene, 2:95-113 (1977)). A population of transformants containing approximately 50,000 individual cDNA clones was grown in one liter of medium overnight and the total plasmid DNA was isolated.

The sequences of two IFN-α clones (IFN-α1 and IFN-α2) have been published (Streuli, M., et al, Science, 209:1343-1347 (1980)). Examination of the DNA sequences of these two clones revealed that the restriction enzyme XhoII would excise a 260 bp fragment from either the IFN-α1 or the IFN-α2 gene (see Figure 1). XhoII was prepared in accordance with the process described by Gingeras, T.R., and Roberts, R.J., J Mol Biol, 118:113-122 (1978).

One mg of the purified total plasmid DNA preparation was digested with XhoII and the DNA fragments were separated on a preparative 6% polyacrylamide gel. DNA from the region of the gel corresponding to 260 bp was recovered by electroelution and recloned by ligation into the BamHI site of the single strand bacteriophage m13:mp7. Thirty-six clones were picked at random, the single stranded DNA isolated therefrom, and the DNA was sequenced. The DNA sequences of four of these clones were homologous to known IFN-α DNA sequences. Clone mp7:α-260, with a DNA sequence identical to IFN-α1 DNA (Streuli, M. et al, Science, 209:1343-1347 (1980)) was chosen as a highly specific hybridization probe for identifying additional IFN-α DNA sequences. This clone is hereinafter referred to as the "260 probe."

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Screening of Genomic DNA Library

In order to isolate other IFN-α gene sequences, a ^{32}P-labelled 260 probe was used to screen a library of human genomic DNA by in situ hybridization. The human gene bank, prepared by Lawn, R.M., et al, Cell, 15:1157-1174 (1978), was generated by partial cleavage of fetal human DNA with HaeIII and AluI and cloned into bacteriophage λ Charon 4A with synthetic EcoRI linkers. Approximately 800,000 clones were screened, of which about 160 hybridized with the 260 probe. Each of the 160 clones was further characterized by restriction enzyme mapping and comparison with the published restriction maps of 10 chromosomal IFN genes (Nagata, S., et al, J Interferon Research, 1:333-336 (1981)). One of the clones, hybrid phage λ4A:α61 containing a 18 kb insert, was characterized as follows. A DNA preparation of λ4A:α61 was cleaved with HindIII, BglII, and EcoRI respectively, the fragments separated on an agarose gel, transferred to a nitrocellulose filter (Southern, E.M., J Mol Biol, 98:503-517 (1977)) and hybridized with ^{32}P-labelled 260 probe. This procedure localized the IFN-α61 gene to a 1.9 kb BglII restriction fragment which was then isolated and recloned, in both orientations, by ligation of the fragment into BamHI cleaved m13:mp7. The two subclones are designated mp7:α61-1 and mp7:α61-2. The -1 designation indicates that the single-stranded bacteriophage contains insert DNA complementary to the mRNA (the minus strand) and the -2 designation indicates that the insert DNA is the same sequence as the mRNA (the plus strand).

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Sequencing of the IFN-α61 Gene

The Sanger dideoxy-technique was used to determine the DNA sequence of the IFN-α61 gene. The strategy employed is diagrammed in Figure 2, the DNA sequence thus obtained is given in Figure 3, and a partial restriction enzyme map of the IFN-α61 gene is illustrated in Figure 4. Unlike many genes from eukaryotic organisms, but analogous to other IFN chromosomal genes which have been characterised, the DNA sequence of this gene demonstrates that it lacks introns. Homology to protein sequence information from these known IFN-α genes made it possible to determine the correct translational reading frame and thus allowed the entire 166 amino acid sequence of IFN-α61 to be predicted from the DNA sequence as well as a precursor segment, or signal polypeptide, of 23 amino acids (Figure 5).

The DNA sequence of the IFN-α61 gene and the amino acid sequence predicted therefrom differ substantially from the other known IFN-α DNA and IFN-α amino acid sequences. In this regard Goeddel, D.V., et al Nature (1981) 290:20-26 discloses the DNA sequence of a partial IFN cDNA clone, designated LeIF-G. The sequence of the partial clone is similar to the 3'-end of the IFN-α61 DNA sequence, except for a nucleotide change in the codon for amino acid 128. As compared to the partial clone the IFN-α61 gene contains additional DNA that codes for the first 33 amino acids of IFN-α61.

Plasmid Preparation and Host Transformation

Assembly of the plasmid for direct expression of the IFN-α61 gene involved replacing the DNA fragment encoding the 23 amino acid signal polypeptide

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of preinterferon with a 120 bp EcoRI/Sau3A promoter fragment (E.coli trp promoter, operator, and trp leader ribosome binding site preceding an ATG initiation codon) and using HindIII site that was inserted, 59 nucleotides 3'- of the TGA translational stop codon, to insert the gene into the plasmid pBW11 (a derivative of pBR322 having a deletion between the HindIII and PvuII sites). The complete DNA sequence of the promoter and gene fragments inserted between the EcoRI and HindIII sites of pBW11 is shown in Figure 6 which also shows the exact location of relevant cloning sites. Details of the construction are described below.

The coding region for mature IFN-α61 has three Sau3A sites, one of which is between codons for amino acids 2 and 3. A synthetic HindIII site was inserted 59 nucleotides 3'- of the coding region and the resulting construct was subjected to a HindIII/partial Sau3A digest. A 560 bp fragment was isolated from the digest. This fragment and a 120 bp EcoRI to Sau3A E.coli promoter fragment were ligated together in a three way directed ligation into the EcoRI to HindIII site of pBW11. The promoter fragment, contained a synthetic HindIII restriction site, ATG initiation codon, the initial cysteine codon (TGT) common to all known IFN-αs, and a Sau3A "sticky end". The ligation mixture was used to transform E.coli MM294 (Backman, K., et al, Proc Natl Acad Sci (USA) 73:4174-4178 (1961)). The desired correct transformation products, 8 out of 24 screened, were identified by restriction enzyme mapping of colonies which hybridized to a ^{32}P-labelled IFN-α genomic fragment. Figure 7 is a diagram of the final expression plasmid obtained, which is designated

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pGW20. Other prokaryotic hosts such as bacteria other than E. coli may, of course, be transformed with this or other suitable constructs to replicate the IFN-α61 gene and/or to produce IFN-α61.

IFN-α61 produced in accordance with the invention is believed to be distinct from the corresponding native protein in several respects. Firstly, because the IFN-α61 gene was expressed by bacterial hosts that utilize N-formyl-methionine and/or methionine to initiate translation, some or all of the bacterially produced IFN-α61 molecules are preceded by an N-formyl-methionine or methionine group. Some of the N-formyl-methionine or methionine groups could be removed by natural *in vivo* bacterial cleavage mechanisms. This would result in a mixture of molecules, some of which would include an initial N-formyl-methionine or methionine and others that would not. All such IFN-α61 molecules, those containing an initial N-formyl-methionine or methionine, those not containing an N-formyl-methionine or methionine and any mixture thereof, are encompassed by the present invention. Secondly, the amino acid residues of the bacterially produced polypeptide are unsubstituted whereas the residues of the native protein may be substituted with sugar groups, ACTH or other moieties. Also, native IFN-α extracts consist of mixtures of various IFN molecules whereas the bacterially produced IFN-α61 is homogeneous; that is, bacterially produced IFN-α61 does not contain functionally related polypeptides. Accordingly, the invention contemplates producing IFN-α61-containing compositions having biological activity that is attributable solely to IFN-α61 and/or said terminal N-formyl-methionine or methionine derivatives thereof.

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Cultivation of Transformants

Bacteria transformed with the IFN-α61 gene may be cultivated in an appropriate growth medium, such as a minimum essential medium, that satisfies the nutritional and other requirements needed to permit the bacteria to grow and produce IFN-α61. If the bacteria are such that the protein is contained in their cytoplasm, the IFN-α61 may be extracted from the cells by lysing the cells such as by sonication and/or treatment with a strong anionic solubilizing agent such as sodium dodecyl sulfate. Further purification of the extract may be achieved by affinity chromatography, electrophoresis, or other protein purification techniques.

Biological Testing of IFN-α61

IFN-α61-containing cell sonicates were tested *in vitro* and found to have the following activities: (1) inhibition of viral replication of vesicular stomatitis virus (VSV) and herpes simplex virus-1 (HSV-1); (2) inhibition of tumor cell growth; (3) inhibition of colony formation by tumor cells in soft agar; (4) activation of natural killer (NK) cells; (5) enhancement of the level of 2',5'-oligoadenylate synthetase (2',5'-A); and (6) enhancement of the double-stranded RNA-dependent protein kinase. The sonicates were active in inhibiting viral infection in both human and other mammalian cells such as hamster, monkey, mouse, and rabbit cells.

The tests show that IFN-α61 exhibits antiviral activity against DNA and RNA viruses, cell growth regulating activity, and an ability to regulate the production of intracellular enzymes and other

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cell-produced substances. Accordingly, it is expected IFN-α61 may be used to treat viral infections with a potential for interferon therapy such as chronic hepatitis B infection, ocular, local, or systemic herpes virus infections, influenza and other respiratory tract virus infections, rabies and other viral zoonoses, arbovirus infections, and slow virus diseases such as Kuru and sclerosing panencephalitis. It may also be useful for treating viral infections in immunocompromised patients such as herpes zoster and varicella, cytomegalovirus, Epstein-Barr virus infection, herpes simplex infections, rubella, and progressive multifocal leukoencephalopathy. Its cell growth regulating activity makes it potentially useful for treating tumors and cancers such as osteogenic sarcoma, multiple myeloma, Hodgkin's disease, nodular, poorly differentiated lymphoma, acute lymphocytic leukemia, breast carcinoma, melanoma, and nasopharyngeal carcinoma. The fact that IFN-α61 increases protein kinase and 2',5'-oligoadenylate synthetase indicates it may also increase synthesis of other enzymes or cell-produced substances commonly affected by IFNs such as histamine, hyaluronic acid, prostaglandin E, tRNA methylase, and aryl hydrocarbon hydrolase. Similarly, it may be useful to inhibit enzymes commonly inhibited by IFNs such as tyrosine amino transferase, glycerol-3-phosphate dehydrogenase glutamine synthetase, ornithine decarboxylase, S-adenosyl-l-methionine decarboxylase, and UDP-N-acetylglucosamine-dolichol monophosphate transferase. The ability of the IFN-α61 to stimulate NK cell activity is indicative that it may also possess other activities such as the abilities to induce macrophage activity and antibody production and to effect cell

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surface alterations such as changes in plasma membrane density or cell surface charge, altered capacity to bind substances such as cholera toxin, concanavalin A and thyroid-stimulating hormone, and change in the exposure of surface gangliosides.

Pharmaceutical compositions that contain IFN-α61 as an active ingredient will normally be formulated with an appropriate solid or liquid carrier depending upon the particular mode of administration being used. For instance, parenteral formulations are usually injectable fluids that use pharmaceutically and physiologically acceptable fluids such as physiological saline, balanced salt solutions, or the like as a vehicle. Oral formulations, on the other hand, may be solid, eg tablet or capsule, or liquid solutions or suspensions. IFN-α61 will usually be formulated as a unit dosage form that contains in the range of 10^4 to 10^7 international units, more usually 10^6 to 10^7 international units, per dose.

IFN-α61 may be administered to humans in various manners such as orally, intravenously, intramuscularly, intraperitoneally, intranasally, intradermally, and subcutaneously. The particular mode of administration and dosage regimen will be selected by the attending physician taking into account the particulars of the patient, the disease and the disease state involved. For instance, viral infections are usually treated by daily or twice daily doses over a few days to a few weeks; whereas tumor or cancer treatment involves daily or multidaily doses over months or years. IFN-α61 therapy may be combined with other treatments and may be combined with or used in association with other chemotherapeutic or chemopreventive agents for providing therapy against viral

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infections, neoplasms, or other conditions against which it is effective. For instance, in the case of herpes virus keratitis treatment, therapy with IFN has been supplemented by thermocautery, debridement and trifluorothymidine therapy.

Modifications of the above described modes for carrying out the invention, such as, without limitation, use of alternative vectors, alternative expression control systems in the vector, and alternative host microorganisms and other therapeutic or related uses of IFN-α61, that are obvious to those of ordinary skill in the biotechnology, pharmaceutical, medical and/or related fields are intended to be within the scope of the following claims.

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Claims

1. A polypeptide having interferon activity and comprising the amino acid sequence:

CysAspLeuProGln ThrHisSerLeuSer AsnArgArgThrLeu MetIleMetAlaGln
MetGlyArgIleSer ProPheSerCysLeu LysAspArgHisAsp PheGlyPheProGln
GluGluPheAspGly AsnGlnPheGlnLys AlaGlnAlaIleSer ValLeuHisGluMet
IleGlnGlnThrPhe AsnLeuPheSerThr LysAspSerSerAla ThrTrpAspGluThr
LeuLeuAspLysPhe TyrThrGluLeuTyr GlnGlnLeuAsnAsp LeuGluAlaCysMet
MetGlnGluValGly ValGluAspThrPro LeuMetAsnValAsp SerIleLeuThrVal
ArgLysTyrPheGln ArgIleThrLeuTyr LeuThrGluLysLys TyrSerProCysAla
TrpGluValValArg AlaGluIleMetArg SerPheSerLeuSer AlaAsnLeuGlnGlu
ArgLeuArgArgLys Glu

2. The polypeptide of claim 1 wherein the polypeptide consists essentially of said amino acid sequence.

3. The polypeptide of claim 1 or 2 wherein the initial cysteine residue of the amino acid sequence is preceded by an N-formyl-methionine group.

4. The polypeptide of claim 1 or 2 wherein the amino acid residues of said sequence are unsubstituted.

5. IFN-α61.

6. A composition having interferon activity and comprising a mixture of:

(a) a polypeptide having the amino acid sequence

CysAspLeuProGln ThrHisSerLeuSer AsnArgArgThrLeu MetIleMetAlaGln
MetGlyArgIleSer ProPheSerCysLeu LysAspArgHisAsp PheGlyPheProGln
GluGluPheAspGly AsnGlnPheGlnLys AlaGlnAlaIleSer ValLeuHisGluMet
IleGlnGlnThrPhe AsnLeuPheSerThr LysAspSerSerAla ThrTrpAspGluThr

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LeuLeuAspLysPhe TyrThrGluLeuTyr GlnGlnLeuAsnAsp LeuGluAlaCysMet
MetGlnGluValGly ValGluAspThrPro LeuMetAsnValAsp SerIleLeuThrVal
ArgLysTyrPheGln ArgIleThrLeuTyr LeuThrGluLysLys TyrSerProCysAla
TrpGluValValArg AlaGluIleMetArg SerPheSerLeuSer AlaAsnLeuGlnGlu
ArgLeuArgArgLys Glu

and;

(b) a polypeptide having said amino acid sequence wherein the initial cysteine residue of the sequence is preceded by an N-formyl-methionine or methionine group.

7. The composition of claim 6 wherein the amino acid residues of said sequence are unsubstituted.

8. A composition having interferon activity comprising a polypeptide having the amino acid sequence:

CysAspLeuProGln ThrHisSerLeuSer AsnArgArgThrLeu MetIleMetAlaGln
MetGlyArgIleSer ProPheSerCysLeu LysAspArgHisAsp PheGlyPheProGln
GluGluPheAspGly AsnGlnPheGlnLys AlaGlnAlaIleSer ValLeuHisGluMet
IleGlnGlnThrPhe AsnLeuPheSerThr LysAspSerSerAla ThrTrpAspGluThr
LeuLeuAspLysPhe TyrThrGluLeuTyr GlnGlnLeuAsnAsp LeuGluAlaCysMet
MetGlnGluValGly ValGluAspThrPro LeuMetAsnValAsp SerIleLeuThrVal
ArgLysTyrPheGln ArgIleThrLeuTyr LeuThrGluLysLys TyrSerProCysAla
TrpGluValValArg AlaGluIleMetArg SerPheSerLeuSer AlaAsnLeuGlnGlu
ArgLeuArgArgLys Glu

or a mixture of said polypeptide and a polypeptide having said sequence wherein the initial cysteine residue is preceded by an N-formyl-methionine or methionine group wherein the interferon activity of the composition is attributable to said polypeptide or to said mixture.

9. A DNA unit consisting of a nucleotide sequence that encodes the polypeptide of claim 1 or 5.

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10. The DNA unit of claim 9 wherein the nucleotide sequence is:

TGT GAT CTG CCT CAG ACC CAC AGC CTG AGT AAC AGG AGG
ACT TTG ATG ATA ATG GCA CAA ATG GGA AGA ATC TCT CCT
TTC TCC TGC CTG AAG GAC AGA CAT GAC TTT GGA TTT CCT
CAG GAG GAG TTT GAT GGC AAC CAG TTC CAG AAG GCT CAA
GCC ATC TCT GTC CTC CAT GAG ATG ATC CAG CAG ACC TTC
AAT CTC TTC AGC ACA AAG GAC TCA TCT GCT ACT TGG GAT
GAG ACA CTT CTA GAC AAA TTC TAC ACT GAA CTT TAC CAG
CAG CTG AAT GAC CTG GAA GCC TGT ATG ATG CAG GAG GTT
GGA GTG GAA GAC ACT CCT CTG ATG AAT GTG GAC TCT ATC
CTG ACT GTG AGA AAA TAC TTT CAA AGA ATC ACT CTC TAT
CTG ACA GAG AAG AAA TAC AGC CCT TGT GCA TGG GAG GTT
GTC AGA GCA GAA ATC ATG AGA TCC TTC TCT TTA TCA GCA
AAC TTG CAA GAA AGA TTA AGG AGG AAG GAA

11. A cloning vehicle that includes the DNA unit of claim 9 or 10.

12. The cloning vehicle of claim 11 wherein the cloning vehicle is a plasmid.

13. The cloning vehicle of claim 11 wherein the cloning vehicle is the plasmid pGW20.

14. A host that is transformed with the cloning vehicle of claim 11 and produces IFN-α61.

15. The host of claim 13 wherein the host is a prokaryote.

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16. The host of claim 14 wherein the host organism is E.coli.

17. A host that is transformed with the cloning vehicle of claim 13 and produces IFN-α61, wherein the host is E.coli.

18. A process for producing IFN-α61 comprising cultivating the host of claim 14 and collecting IFN-α61 from the resulting culture.

19. A process of producing IFN-α61 comprising cultivating the host organism of claim 16 and collecting IFN-α61 from the resulting culture.

20. A process for producing IFN-α61 comprising cultivating the host organism of claim 17 and collecting IFN-α61 from the resulting culture.

21. A pharmaceutical composition comprising an effective amount of the polypeptide of claim 1, 2 or 5 admixed with a pharmaceutically acceptable vehicle or carrier.

22. A pharmaceutical composition comprising an effective amount of the composition of claim 6 or 8 admixed with a pharmaceutically acceptable vehicle or carrier.

23. A method of providing interferon therapy to a human comprising administering a therapeutically effective amount of the polypeptide of claim 1, 2 or 5 to said human.

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24. A method of providing interferon therapy to a human comprising administering a therapeutically effective amount of the composition of claim 6 or 8 to said human.

25. The method of claim 23 wherein the therapy is for treating a viral infection, providing cell growth regulation, or regulating the production of a cell-produced substance.

26. The method of claim 24 wherein the therapy is for treating a viral infection, providing cell growth regulation, or regulating the production of a cell-produced substance.

27. A method of providing antiviral therapy to a mammal comprising administering a viral infection inhibiting amount of the polypeptide of claim 1, 2 or 5 to the mammal.

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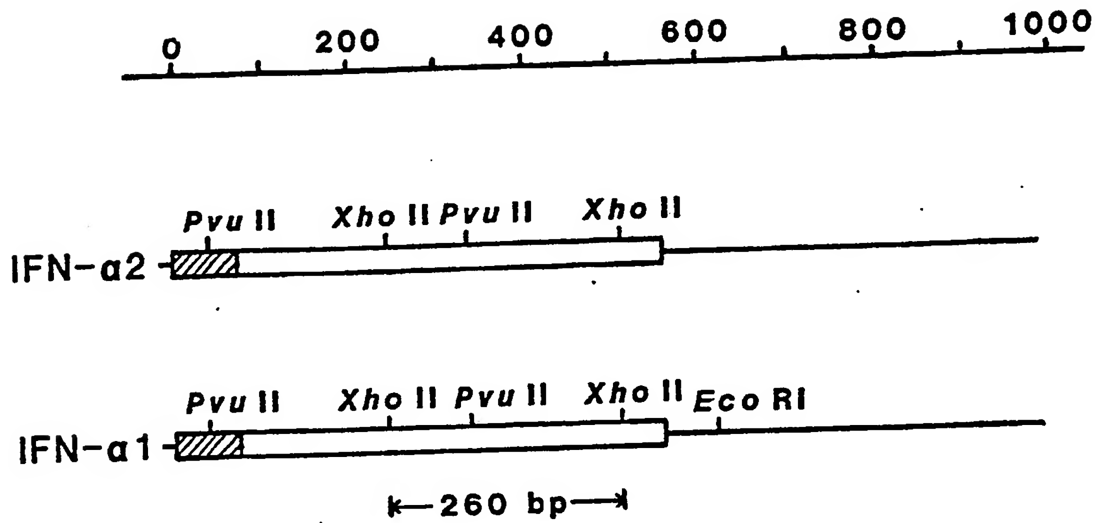

Figure 1

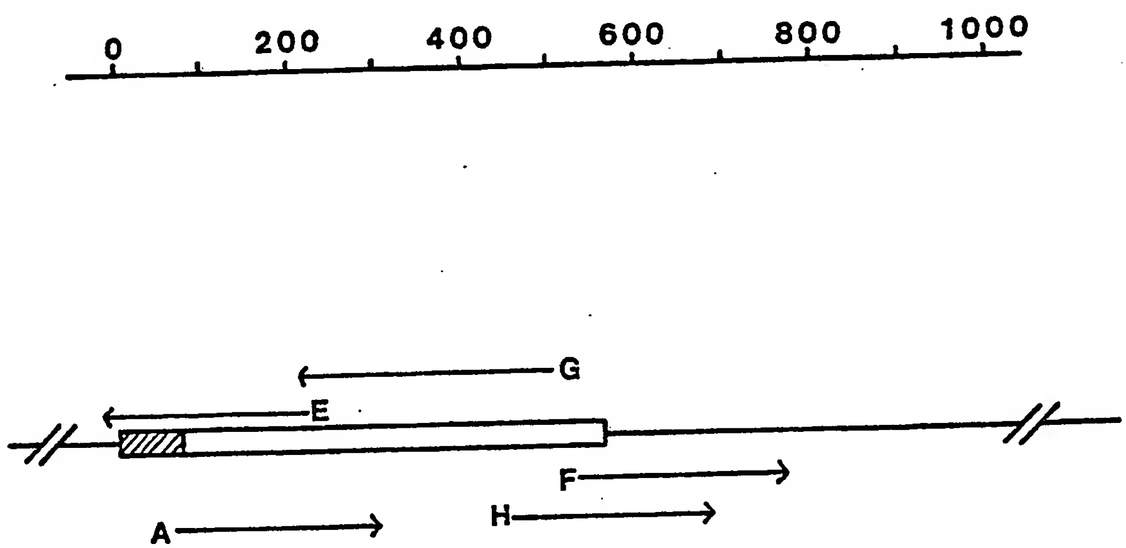

Figure 2

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        10         20         30         40         50         60
AGATCTGTGC ACAAAACAAG GTCTTCAGAG AAGAGCCCAA GGTTCAGGGT CACTCAATCT
TCTAGACACG TGTTTTGTTC CAGAAGTCTC TTCTCGGGTT CCAAGTCCCA GTGAGTTAGA
        70         80         90        100        110        120
CAACAGCCCA GAAGCATCTG CAACCTCCCC AATGGCCTTG CCCTTTGTTT TACTGATGGC
GTTGTCGGGT CTTCGTAGAC GTTGGAGGGG TTACCGGAAC GGGAAACAAA ATGACTACCG
       130        140        150        160        170        180
CCTGGTGGTG CTCAACTGCA AGTCAATCTG TTCTCTGGGC TGTGATCTGC CTCAGACCCA
GGACCACCAC GAGTTGACGT TCAGTTAGAC AAGAGACCCG ACACTAGACG GAGTCTGGGT
       190        200        210        220        230        240
CAGCCTGAGT AACAGGAGGA CTTTGATGAT AATGGCACAA ATGGGAAGAA TCTCTCCTTT
GTCGGACTCA TTGTCCTCCT GAAACTACTA TTACCGTGTT TACCCTTCTT AGAGAGGAAA
       250        260        270        280        290        300
CTCCTGCCTG AAGGACAGAC ATGACTTTGG ATTTCCTCAG GAGGAGTTTG ATGGCAACCA
GAGGACGGAC TTCCTGTCTG TACTGAAACC TAAAGGAGTC CTCCTCAAAC TACCGTTGGT
       310        320        330        340        350        360
GTTCCAGAAG GCTCAAGCCA TCTCTGTCCT CCATGAGATG ATCCAGCAGA CCTTCAATCT
CAAGGTCTTC CGAGTTCGGT AGAGACAGGA GGTACTCTAC TAGGTCGTCT GGAAGTTAGA
       370        380        390        400        410        420
CTTCAGCACA AAGGACTCAT CTGCTACTTG GGATGAGACA CTTCTAGACA AATTCTACAC
GAAGTCGTGT TTCCTGAGTA GACGATGAAC CCTACTCTGT GAAGATCTGT TTAAGATGTG
       430        440        450        460        470        480
TGAACTTTAC CAGCAGCTGA ATGACCTGGA AGCCTGTATG ATGCAGGAGG TTGGAGTGGA
ACTTGAAATG GTCGTCGACT TACTGGACCT TCGGACATAC TACGTCCTCC AACCTCACCT
       490        500        510        520        530        540
AGACACTCCT CTGATGAATG TGGACTCTAT CCTGACTGTG AGAAAATACT TTCAAAGAAT
TCTGTGAGGA GACTACTTAC ACCTGAGATA GGACTGACAC TCTTTTATGA AAGTTTCTTA
       550        560        570        580        590        600
CACTCTCTAT CTGACAGAGA AGAAATACAG CCCTTGTGCA TGGGAGGTTG TCAGAGCAGA
GTGAGAGATA GACTGTCTCT TCTTTATGTC GGGAACACGT ACCCTCCAAC AGTCTCGTCT
       610        620        630        640        650        660
AATCATGAGA TCCTTCTCTT TATCAGCAAA CTTGCAAGAA AGATTAAGGA GGAAGGAATG
TTAGTACTCT AGGAAGAGAA ATAGTCGTTT GAACGTTCTT TCTAATTCCT CCTTCCTTAC
       670        680        690        700        710        720
AAAACTGGTT CAACATCGAA ATGATTCTCA TTGACTAGTA CACCATTTCA CACTTCTTGA
TTTTGACCAA GTTGTAGCTT TACTAAGAGT AACTGATCAT GTGGTAAAGT GTGAAGAACT
       730        740        750        760        770        780
GTTCTGCCGT TTCAAATATT AATTTCTGCT ATATCCATGA CTTGAGTTGA ATCAAAATTT
CAAGACGGCA AAGTTTATAA TTAAAGACGA TATAGGTACT GAACTCAACT TAGTTTTAAA
       790        800        810        820        830
TCAAACGTTT CACACGTGTT AAGCAACACT TCTTTAGCTC CACAGGGACA AAA
AGTTTGCAAA GTGTGCACAA TTCGTTGTGA AGAAATCGAG GTGTCCCTGT TTT
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Figure 3

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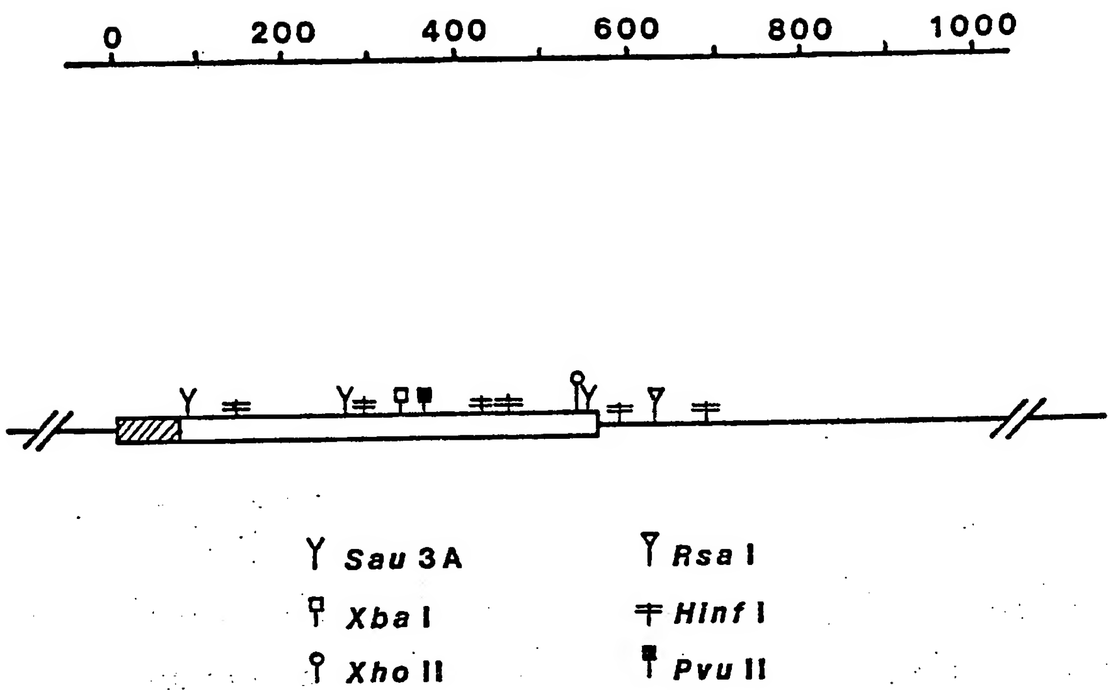

Figure 4

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1
Met Ala Leu Pro Phe Val Leu Leu Met Ala Leu Val Val Leu Asn Cys Lys Ser Ile Cys
ATG GCC TTG CCC TTT GTT TTA CTG ATG GCC CTG GTG GTG CTC AAC TGC AAG TCA ATC TGT

21
Ser Leu Gly Cys Asp Leu Pro Gln Thr His Ser Leu Ser Asn Arg Arg Thr Leu Met Ile
TCT CTG GGC TGT GAT CTG CCT CAG ACC CAC AGC CTG AGT AAC AGG AGG ACT TTG ATG ATA

41
Met Ala Gln Met Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly
ATG GCA CAA ATG GGA AGA ATC TCT CCT TTC TCC TGC CTG AAG GAC AGA CAT GAC TTT GGA

61
Phe Pro Gln Glu Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu
TTT CCT CAG GAG GAG TTT GAT GGC AAC CAG TTC CAG AAG GCT CAA GCC ATC TCT GTC CTC

81
His Glu Met Ile Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp
CAT GAG ATG ATC CAG CAG ACC TTC AAT CTC TTC AGC ACA AAG GAC TCA TCT GCT ACT TGG

101
Asp Glu Thr Leu Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu
GAT GAG ACA CTT CTA GAC AAA TTC TAC ACT GAA CTT TAC CAG CAG CTG AAT GAC CTG GAA

121
Ala Cys Met Met Gln Glu Val Gly Val Glu Asp Thr Pro Leu Met Asn Val Asp Ser Ile
GCC TGT ATG ATG CAG GAG GTT GGA GTG GAA GAC ACT CCT CTG ATG AAT GTG GAC TCT ATC

141
Leu Thr Val Arg Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser
CTG ACT GTG AGA AAA TAC TTT CAA AGA ATC ACT CTC TAT CTG ACA GAG AAG AAA TAC AGC

161
Pro Cys Ala Trp Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ala Asn
CCT TGT GCA TGG GAG GTT GTC AGA GCA GAA ATC ATG AGA TCC TTC TCT TTA TCA GCA AAC

181
Leu Gln Glu Arg Leu Arg Arg Lys Glu
TTG CAA GAA AGA TTA AGG AGG AAG GAA

Figure 5

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1
GAA TTC CGA CAT CAT AAC GGT TCT GGC AAA TAT TCT GAA ATG AGC TGT TGA CAA TTA ATC
 Eco RI
                                                                        Met Cys
61
ATC GAA CTA GTT AAC TAG TAC GCA AGT TCA CGT AAA AAG GGT ATC GAT AAG CTT ATG TGT

121
Asp Leu Pro Gln Thr His Ser Leu Ser Asn Arg Arg Thr Leu Met Ile Met Ala Gln Met
GAT CTG CCT CAG ACC CAC AGC CTG AGT AAC AGG AGG ACT TTG ATG ATA ATG GCA CAA ATG
Sau 3A
181
Gly Arg Ile Ser Pro Phe Ser Cys Leu Lys Asp Arg His Asp Phe Gly Phe Pro Gln Glu
GGA AGA ATC TCT CCT TTC TCC TGC CTG AAG GAC AGA CAT GAC TTT GGA TTT CCT CAG GAG

241
Glu Phe Asp Gly Asn Gln Phe Gln Lys Ala Gln Ala Ile Ser Val Leu His Glu Met Ile
GAG TTT GAT GGC AAC CAG TTC CAG AAG GCT CAA GCC ATC TCT GTC CTC CAT GAG ATG ATC

301
Gln Gln Thr Phe Asn Leu Phe Ser Thr Lys Asp Ser Ser Ala Thr Trp Asp Glu Thr Leu
CAG CAG ACC TTC AAT CTC TTC AGC ACA AAG GAC TCA TCT GCT ACT TGG GAT GAG ACA CTT

361
Leu Asp Lys Phe Tyr Thr Glu Leu Tyr Gln Gln Leu Asn Asp Leu Glu Ala Cys Met Met
CTA GAC AAA TTC TAC ACT GAA CTT TAC CAG CAG CTG AAT GAC CTG GAA GCC TGT ATG ATG

421
Gln Glu Val Gly Val Glu Asp Thr Pro Leu Met Asn Val Asp Ser Ile Leu Thr Val Arg
CAG GAG GTT GGA GTG GAA GAC ACT CCT CTG ATG AAT GTG GAC TCT ATC CTG ACT GTG AGA

481
Lys Tyr Phe Gln Arg Ile Thr Leu Tyr Leu Thr Glu Lys Lys Tyr Ser Pro Cys Ala Trp
AAA TAC TTT CAA AGA ATC ACT CTC TAT CTG ACA GAG AAG AAA TAC AGC CCT TGT GCA TGG

541
Glu Val Val Arg Ala Glu Ile Met Arg Ser Phe Ser Leu Ser Ala Asn Leu Gln Glu Arg
GAG GTT GTC AGA GCA GAA ATC ATG AGA TCC TTC TCT TTA TCA GCA AAC TTG CAA GAA AGA

601
Leu Arg Arg Lys Glu ***
TTA AGG AGG AAG GAA TGA AAA CTG GTT CAA CAT CGA AAT GAT TCT CAT TGA CTA GTA CAC

661
ATA AGC TT
  Hind III
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Figure 6

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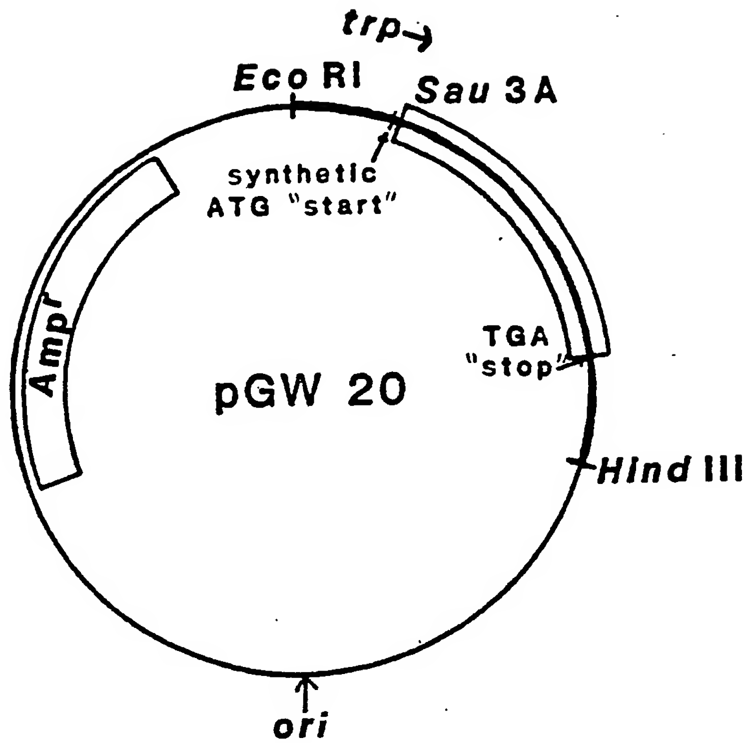

IFN-α61 Expression Plasmid

Figure 7

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INTERNATIONAL SEARCH REPORT

International Application No PCT/US 83/00034

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [8]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[3]: C 12 N 15/00; C 07 C 103/52; C 12 P 21/02; A 61 K 45/02; C 07 M 21/04; C 12 N 1/20 // C 12 R 1/19

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
IPC[3]	C 07 C; C 12 N; A 61 K; C 12 R

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	Nature, volume 290, 5 March 1981, D. Goeddel et al.: "The structure of eight distinct cloned human leukocyte interferon C DNA's", pages 20-26, see the entire document --	1,4,8-12
Y	Nature, volume 287, 2 October 1980, D.Goeddel et al.: "Human leukocyte interferon produced by E.Coli is biologically active", pages 411-416, see the entire document (cited in the application) --	1,4,8-12
Y	Proc.Natl.Acad.Sci, volume 78, no. 9, September 1981 (US) "DNA sequence of a major human leukocyte interferon gene", pages 5435-5439, see the entire document -- ./.	1,4,8-10

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
21st April 1983	11 MAI 1983
International Searching Authority [1]	**Signature of Authorized Officer [20]**
EUROPEAN PATENT OFFICE	G.L.M. Kruydenberg

Form PCT/ISA/210 (second sheet) (October 1981)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	Science, volume 209, 19 September 1980, M.Streuli et al.: "At least three human type alpha interferons: Structure of alpha 2", pages 1343-1347, see the entire document (cited in the application)	1,4,8-10
	--	
Y	EP, A, 0042246 (CANCER INSTITUTE OF JAPANESE FOUNDATION FOR CANCER RESEARCH) 23 December 1981, see claims 1-8	1,2,4,8-12

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers °°) because they relate to subject matter [12] not required to be searched by this Authority, namely:

°°) 23-27 (PCT Rule 39.1iv)

2. [ ] Claim numbers ______, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.